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23. A method according to claim 9 wherein the immunoconjugate protein is constructed as a dimer of two identical chains, each having an effector domain and a targeting domain.

24. A method according to claim 9 wherein the effector domain is the Fc region of an IgG1 immunoglobulin.

REMARKS

Claims 1 to 20 were pending in this application in its published PCT format. Claims 1 and 9 were amended to substitute functional language for the structural language describing the effector domain originally presented, particularly pointing out that that portion of immunoconjugates of the invention induce a cytolytic response or cytotoxic effect against a targed cell, as set out in the abstract, and that the targeting domain of immunoconjugates of the invention exhibit reduced blood coagulation activity as illustrated in the Examples. Claims 6, 14, 15, 19 and 20 were amended to convert them from multiple dependent claims to dependent claims to save fees. Claim 13 was amended to include methods of treating atherosclerosis, as set out in the specification on page 12 at line 9 and in the abstract. Claims 21 to 24 were added to provide dependent claims reciting the structural limitations set out in originally presented claims 1 and 9. As amended, the claim set consists of 24 claims, three of which are independent and the others are dependent, as set out in the claim fee schedule accompanying this filing.

The specification was amended to include the PCT related application data and the abstract that appeared on the cover sheet of WO 01/02439 when it was published on 11 January 2001.

This invention relates to immunoconjugates useful in the treatment of pathological conditions characterized by neovascularization comprising an immunoconjugate protein having an effector domain which can induce a cytolytic or cytotoxic immune

response against a targeted cell, conjugated to a targeting domain which is a mutant form of factor VII that binds to tissue factor and exhibits reduced blood coagulation activity. In some embodiments, immunoconjugates of the invention are constructed as a dimer of two identical chains, each having an effector domain and a targeting domain. In some preferred embodiments, the effector domain comprises the Fc region of an IgG1 immunoglobulin.

The immunoconjugate constructs applicants claim are structurally and functionally different from the molecules disclosed in the references cited in the International Search Report during the pendency of the PCT application. The molecules described by Thorpe and Edgington in U.S. Pat. No. 5,877,289, for example, differ from the claimed molecules of this invention in several ways. Thorpe and Edgington's pharmacologic molecules are bispecific antibodies composed of two scFv antibody fragments, one for the targeting domain and the other for the effector domain (U.S. 5,877,289, column 3, lines 61 to 65, Figure 5, and the Examples). Applicant's immunoconjugates, in contrast, does not induce blood clots and are composed of factor VII as the targeting domain and the Fc portion of an immunoglobulin as the effector domain. The mechanisms of action of the two pharmacologic molecules are also unrelated. The Thorpe and Edgington molecule is designed to block the blood supply to a growing tumor by inducing blood clots in the tumor vasculature (column 16, lines 36 to 37). Applicants' molecule, in contrast, induces an immune attack that specifically destroys the tumor vasculature. Thorpe and Edgington require a scFv antibody that binds specifically to an antigen on the tumor vasculature, but they have not identified the antigen or produced the antibody. Applicants use factor VII, which is the natural ligand for tissue factor, to bind specifically to tissue factor, which is expressed on the endothelium of the tumor vasculature. The affinity of fVII for TF is at least two orders of magnitude stronger than the strongest affinity of a scFv antibody against its target. It is significant and telling to note that the powerful efficacy of Applicants' strategy has been demonstrated in animal models of human cancers that can be translated directly to the clinic. A limited efficacy of the Thorpe and Edgington

strategy has been demonstrated in an artificial system that has no direct relevance to the clinic.

A direct comparison of the composition and function of the immunoconjugates of this invention with and the scFv-tTF complexes described by Thorpe and Edgington summarize the differences:

	Targeting domain	Function
Applicants	Human factor VII with a mutation that inhibits blood coagulation	Natural ligand that binds tightly and specifically to human tissue factor
Thorpe, *et al.*	scFv antibody	Murine antibody that can bind to an antigen on the tumor vascular endothelium
	Effector domain	**Function**
Applicants	Fc region of a human IgG1 immunoglobulin	Induces a cytolytic immune response against cells that bind the icon, causing destruction of the tumor vasculature
Thorpe, *et al.*	scFv antibody	Murine antibody that can bind to tissue factor, initiating blood coagulation

Note that Thorpe and Edgington do not describe or use factor VII or factor VII mutants. Both the targeting domain and the effector domain of the Thorpe and Edgington immunoconjugates are scFv antibodies, and the effector domain binds to a mutated tissue factor, and not to a mutated factor VII to initiate blood coagulation. The Thorpe and Edgington molecules are structurally and functionally completely different from what Applicant claims.

The factor VII mutant (Ser 344 to Ala 344) described by Berkner, *et al.*, in U.S. Pat. No. 5,861,374 was designed to interrupt the blood coagulation cascade for treatment of coagulation-related disorders (abstract, lines 1 to 3). The factor VII mutant described and claimed by Applicant (Lys 341 to Ala 341) was designed as the targeting domain of an

immunoconjugate molecule for treatment of angiogenic-related diseases. Thus, the composition and application of the Berkner, *et al.*, factor VII molecule and Applicants' immunoconjugate molecule are structurally and functionally distinct.

In *Proc. Natl. Acad. Sci. USA 96*: 1627-1632 (1999), Wang, *et al.*, describe an immunoconjugate molecule composed of a scFv targeting domain conjugated to a human Fc effector domain (abstract, lines 1 to 3). This molecule is one type of immunoconjugate for the treatment of angiogenic-related diseases, and so the paper provides enablement for the type of neovascular-targeted immunoconjugates claimed, but it does not suggest the structure of the immunoconjugate constructs claimed in this patent application. Applicants' targeting domain is human factor VII with a mutation that inhibits blood coagulation and the effector domain induces a cytolygic immune response that destroys tumor vasculature. Neither feature is disclosed by Wang, *et al.*, who describe immunoconjugates having a scFv targeting domain that targets the tumor and not the tumor vasculature. None of the references anticipate or suggest using a human factor VII mutant in the targeting domain of an immunoconjugate.

The claimed invention provides new immunoconjugate constructs and methods for their use. In one embodiment, applicants employ factor VII as the natural ligand for TF and the Fc region as the effector domain of an antibody in a combination that provides immunoconjugates having remarkable anti-tumor efficacy. No precedent exists for the use of factor VII, the natural ligand for TF, to target the tumor neovasculature for destruction by the endogenous immune system. The use of TF as the specific natural target for the tumor vasculature, and the use of the factor VII immunoconjugate as the targeting molecule, are entirely novel. A subsequent paper by Applicants (*Proc. Nat. Acad. Sci. USA 98*: 12180-12185 (2001), attached hereto, has convincingly demonstrated the efficacy and safety of using the factor VII immunoconjugate for cancer immunotherapy. Applicants therefore request early and favorable consideration of the amended claims.

If the undersigned can advance the prosecution of this application in any way, the Examiner is invited to call at the number listed below.

Respectfully submitted,

on 31 December 2001 by

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Marked Up Version of Amendments Required by 37 C.F.R. § 1.121

Amendments to the specification:

This application claims priority benefit of U.S. Serial number 60/142,161, filed July 1, 1999 and PCT/US00/16481, filed 14 June 2000.

Amendments to the Claims:

1 (Amended). A composition useful in the treatment of pathological conditions characterized by neovascularization comprising an immunoconjugate protein [constructed as a dimer of two identical chains, each] having an effector domain which [is the Fc region of an IgG1 immunoglobulin] can induce a cytolytic immune response or cytotoxic effect against a targeted cell, conjugated to a targeting domain which is a mutant form of factor VII that binds to tissue factor [but does not initiate] and has reducd blood coagulation activity.

6 (Amended). A composition according to [any of claims] claim 1 [to 5] wherein the immunoconjugate protein is encoded as a secreted molecule in an expression vector.

9 (Amended). A method for treating a disease associated with neovascularization, which comprises administering to a patient having the disease an effective amount of at least one type of immunoconjugate protein having an effector domain which can induce a cytolytic immune response or cytotoxic effect against a targeted cell, [comprising the Fc region of an IgG1 immunoglobulin] conjugated to a targeting domain comprising a mutant form of factor VII that binds to tissue factor [but does not initiate] and has reduced blood clotting activity.

13 (Amended). A method according to claim 9 which is a treatment for a disease selected from the group consisting of cancer involving a vascularized tumor, rheumatoid arthritis, [and] the exudative form of macular degeneration, and atherosclerosis.

14 (Amended). A method according to [any of claims] claim 9 [to 13] wherein the patient is treated by administration of an immunoconjugate protein in a pharmaceutically acceptable carrier.

15 (Amended). A method according to [any of claims] claim 9 [to 13] wherein the patient is treated by administration of a replication-deficient adenoviral vector or an adeno-associated vector carrying a cDNA encoding a secreted form of one or more types of immunoconjugate protein.

19 (Amended). A method according to [claims] claim 17 [or 18] wherein the patient is treated by adminstering the immunoconjugate in a pharmaceutically acceptable carrier.

20 (Amended). A method according to [claims] claim 17 [or 18] wherein the patient is treated by administering a replication-deficient adenoviral vector carrying a cDNA encoding a secreted form of one or more types of the immunoconjugate protein.